

PRELIMINARY AMENDMENT

Applicant(s): High et al.

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Serial No.: 09/877,220


Confirmation No.: 8535

Filed: 8 June 2001


For: METHODS FOR TREATING NEUROPATHOLOGICAL STATES AND NEUROGENIC
INFLAMMATORY STATES AND METHODS FOR IDENTIFYING COMPOUNDS USEFUL THEREIN

neurons include neurons in the spinal cord, for instance neurons in the dorsal horn and motor horn, the brain, for instance neurons in the basal forebrain and hippocampus, peripheral neurons, for instance dorsal root ganglia.

Please replace the paragraph beginning at page 10, line 17, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

 Examples of other cells useful in the present invention include, for instance, cultured synovial sarcoma cells, preferably rat, mouse, or human, more preferably human. An example of a cultured human synovial sarcoma cell is SW982 (ATCC HTB-93). Examples of *in vivo* cells include synovial cells lining a knee joint of a subject, preferably a human.

Please replace the paragraph beginning at page 11, line 23, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

 Preferred examples of compounds that can be used in some aspects of the present invention include tyrosine kinase inhibitors. Tyrosine kinase inhibitors are known in the art and include, for instance, Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one; 4',5,7-trihydroxy-isoflavone, Catalog Number G-103 from RBI, Natick, Massachusetts), Lavendustin A (5-Amino-[N-2,5-dihydroxybenzyl)-N'-2-hydroxybenzyl]salicylic acid, Catalog Number 428150 from Calbiochem, La Jolla, California), and K252a (Catalog Number 420298, from Calbiochem, La Jolla, California). Whether a compound is a tyrosine kinase inhibitor can be determined using methods known in the art (see, for instance, Akiyama et al., *J. Biol. Chem.*, 262, 5592-5595 (1987) and Ohmichi et al., *Biochemistry*, 31(16:4034-4039 (1992)). Preferably, a tyrosine kinase

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inhibitor useful in the present invention decreases phosphorylation of NR1 receptor. Without being limiting, it is expected that a specific tyrosine kinase mediates the translocation of NR1 subunit, and that this specific tyrosine kinase will be a member of one of the known families of tyrosine kinases, for instance, the Src or Jak families. Inhibitors are available that specifically inhibit individual members of the known families of tyrosine kinases. Accordingly, it is expected that specific tyrosine kinase inhibitors will be useful in the methods of the present invention. Alternatively, it is expected that other useful compounds include tyrosine phosphatases and serine/threonine phosphatases. In other aspects of the present invention, preferred examples of compounds that can be used include tyrosine kinases, tyrosine phosphatase inhibitors, or serine/threonine phosphatase inhibitors where increases in NR1 subunit would be advantageous such as for improving memory or slowing the aging process.

Please replace the paragraph beginning at page 13, line 4, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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Activation of a cell *in vivo* is typically accomplished by using an animal model that can be used for investigating conditions that result from sensitization of cells or from increased concentrations of glutamate. Without intending to be limiting, such conditions include neuropathological states and neurogenic inflammatory states. Animal models for studying these conditions are known in the art and can be used in the methods of the present invention. Models for the study of pain include those approved by the International Association for the Study of Pain. A preferred animal model for identifying compounds that alter the distribution of NR1 subunits in a cell is the rat arthritis model described in Example 1. The rat arthritis model is a commonly accepted model for the study

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of pain and arthritis in humans. Other animal models (for instance, using cat, monkey, or rabbit as the animal) are also commonly accepted models for these human conditions (see, e.g., Neugebauer & Schaible, *Agents and Actions*, 25, 234-236 (1988) and O'Byrne et al., *Arthritis and Rheumatism*, 33, 1023-1028 (1990)). To study pain in this model, cells in the spinal cord are exposed to a compound for a period of time, and then a knee of the animal is exposed to a stimulus that evokes persistent pain. Methods of evoking persistent pain are known in the art. After a period of time the responsiveness of the animal to an innocuous or noxious stimulus is evaluated using methods known in the art. A compound that causes an animal to have reduced primary allodynia or secondary allodynia, or reduced primary hyperplasia or secondary hyperplasia compared to an animal that has not received the compound indicates that the distribution of NR1 subunits in the spinal cord is altered. This model may also be used to study arthritis. A compound can be injected into the synovial space of a knee joint, and then the knee exposed to a stimulus that evokes arthritis. After a period of time, the responsiveness of the animal to movements in the working range of the joint are evaluated. A compound that causes an animal to have reduced response time when its foot is touched, that is, the animal overreacts, compared to an animal that has not received the compound indicates that the distribution of NR1 subunits in the cells lining the synovial space, and/or the production of $\text{TNF}\alpha$ by the cells is altered. Neuropathological states and neurogenic inflammatory states can also be experimentally recreated *in vivo* by injecting a glutamate receptor agonist into a space containing neurons (for instance, the spinal cord) or other cells (for instance, the synovial space present in a joint) that include a glutamate receptor.

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Please replace the paragraph beginning at page 24, line 6, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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a. *Acute induction with kaolin/carrageenan.* An acute inflammatory response restricted to the knee joint can be induced by the injection of 3% kaolin and 3% carrageenan (in sterile saline; 0.1 ml; pH 7.4) into the joint cavity while the animal is briefly anesthetized with sodium methohexital (Brevital, 60 mg/kg, i.p.). Kaolin and carrageenan were obtained from Fisher Scientific, St. Louis, Missouri. The knee joint is flexed manually until the rat awakes (approximately 5-10 minutes). In this arthritis model in the awake rat, localized joint swelling, as well as limping and guarding of the limb, are well developed at 4 hours (Sluka et al., *Pain*, 59, 95-100 (1994)) when behavioral testing begins.

Please replace the paragraph beginning at page 24, line 29, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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Fifty-five rats were used for behavioral studies. Four hours after induction of the arthritis, the joint is swollen and increased withdrawal responses to radiant heat and spontaneous guarding of the limb were noted. The increased responsiveness to noxious stimuli indicates the presence of secondary hyperalgesia. Testing of paw withdrawal latency (PWL) to radiant heat on the footpad using the Hargreaves method (Hargreaves et al., *Pain*, 32, 77-88 (1988)), as a measure of secondary hyperalgesia (away from the primary site of injury indicative of central pain) reveals that the acute inflammation renders the hindlimb more sensitive to heat stimuli (Sluka et al., *Pain*, 59, 95-100 (1994)). Briefly, animals are placed in small Lucite cubicles on a glass top table cooled with a fan and allowed to accommodate for 30 minutes prior to testing. A hand-held metal box focusing a high intensity light

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through an aperture (1 cm X 0.8 cm) is used to apply radiant heat through the glass to the plantar surface of the hindpaw until the animal lifts its foot. Radiant heat was applied to the plantar surface of the hindpaw until the rat lifted its paw. The time which it took for this to occur was considered the PWL response time. Both paws were tested independently at five minute intervals for a total of five trials. A mean of these five reading was used as PWL response for each time point. Testing was done by the same observer for each test, and the observer was blinded to the test groups under study. A decrease in PWL occurred on the side ipsilateral to the inflamed knee 4 hours after the induction of acute arthritis and was linearly correlated with the increase in joint swelling. In the experimental rats the PWL was measured before administration of drug or vehicle (baseline) and after the drug or vehicle had been infused for 1.5 hours at which time kaolin and carrageenan was injected into the knee joint. The final measurement for PWL was at 4 hours after induction of arthritis. A decrease of the PWL to noxious radiant heat in a rat with knee joint inflammation is indicative of secondary hyperalgesia.

Please replace the paragraph beginning at page 27, line 17, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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Reflexive withdrawal of the paw (PWL) to radiant heat is known to be reduced from baseline 4 hours after induction of knee joint inflammation in this arthritis model in rats (Sluka et al., *Neurosci. Lett.*, 145:141-144 (1992)). Comparisons were made with baseline and between treatment groups at 4 hours after joint inflammation (ANOVA). The behavioral studies demonstrated that pre-treatment with the protein tyrosine kinase inhibitor, Genistein, significantly attenuates the inflammation-induced decrease in PWL in response to radiant heat

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indicative of secondary hyperalgesia and a central sensitization state in the central nervous system in this arthritis model. Secondary hyperalgesia requires sensitization of various portions of the neuronal circuitry in addition to the local spinal reflex loop. Secondary hyperalgesia which can be measured is a manifestation of central sensitization. Thus central sensitization is sensitization of structures of the pain circuitry as opposed to sensitization of the peripheral nerve of the spinal loop.
